STUDIES ON THE BLOOD OF INSECTS.

By Richard A. Muttkowski, Moscow, Idaho.

II. The Structural Elements of the Blood.*

1. Materials and Methods.

In the study of the blood of insects several important factors must be considered. One is that the blood courses in a haemocoel and is in direct contact with the various tissues, which pour their products such as secretions, wastes, and histolytic remnants, into the blood. Histolysis and histogenesis are constantly going on during the larval and pupal period and one may find embryonic cells and degenerating tissues in the blood stream. Indeed, the corpuscles themselves are much like embryonic cells and are able to take up various functions as the need arises.

As a result, conditions as found in the larvae are generally somewhat confusing and it is advisable to check up the corpuscles of the larvae, if possible, with those of the adults. For my personal studies, I have held to the criterion that the blood of long-lived adults should give the best information as to what the real corpuscular elements consist of; especially, if these adults were studied a month or more following the final ecdysis, or after hibernation, when all foreign elements are removed and only the true corpuscles remain. If the corpuscles of such adults are examined first, recognition of the larval cells is greatly facilitated.

The manner of drawing insect blood is also of importance. For larvae and pupae a puncture into the abdomen with a sharp triangular needle results in good-sized drops; few tissue elements, such as adipose cells, oenocytes, and muscle fibers, are ever found in such drops. For adults a cut across the base of one of the wings opens the alar channels and yields copious and "uncontaminated" material. These procedures are more satis-

* Contribution from the Zoological Laboratory, University of Idaho, Moscow, Idaho.

factory than slitting the abdominal walls; by the latter means too many tissue elements are loosened and mingled with the corpuscles.

As regards methods, I have found smear preparations very unsatisfactory. Insect blood does not lend itself favorably for smears; its viscosity causes clumping and undue distortion of the corpuscles, while the gelatin interferes with precise staining. However, I have obtained excellent smears by the following method: A drop of blood is spread somewhat on a slide and placed at once in a moist chamber with excess of moisture. The blood may also be thinned by adding a drop or two of 1 per cent. salt solution. After fifteen minutes to half an hour the drop is decanted, leaving the corpuscles as a white residue. If there is a gelatin coagulum, careful washing will remove it. Flood with a fixative, or immerse in it, and follow by the usual procedures. Such slides show the corpuscles relaxed and in the midst of their activities. Smears can also be stained without use of a fixative, as the stain takes the place of the latter, and taken through the alcohols to balsam or gum-dammar for permanent mounts.

For the study of the functions bulk fixation followed by sectioning, but particularly *in vitro* studies and *direct* staining methods were found of advantage. *In vitro* studies can be made with sealed drops, that is, where the cover-slips have been margined with vaseline to prevent evaporation. With careful manipulation of lights, the movements of the corpuscles can be readily observed. The nuclei, larger pseudopodia, and cytoplasmic bodies, and sometimes fibrin threads, can be recognized without difficulty. The hind wings of Coleoptera, Hemiptera, Orthoptera, and smaller Lepidoptera can be similarly employed. For finer details, however, staining is necessary.

Previous to staining a certain technique is advisable to induce full activity on the part of the corpuscles. Slides with covered drops are placed in moist chambers for fifteen minutes to half an hour to permit " relaxation." A drop of stain is then placed at the edge of the cover-slip and allowed to filter in. The stain acts as a mild fixative and does not cause any distortion. It penetrates only a portion of the drop and becomes diluted as it diffuses inward. This is a distinct advantage, since the intensity of the stain is thus graduated toward the center of the slide.

For *direct staining* over forty stains were tried; but methylene blue, methyl green, Pianese methylene blue-eosin, methyl violet,

and picrohematoxylin yielded the best result. Methylene blue was used 0.5 per cent. in 1 per cent. salt solution; it is good for nuclear and cytoplasmic details, but does not show the finer pseudopodia very well, and fibrin not at all. Acidulated methyl green in a 1.0 per cent. aqueous solution reacts similarly to methylene blue, but is inferior to it. Methyl violet, in a 0.1 per cent. solution—stronger solutions stain too deeply—penetrates with great rapidity, bringing out the nuclei, pseudopodia, fibrin, fat bubbles, and various other bodies with considerable sharpness. It is good for general purposes, but not for the finer details. Furthermore, the stain may cause a copious protein precipitate which tends to obscure the corpuscles. It is best used as a check on other stains.

Pianese's methylene blue-eosin is methylene blue saturated in a saturated borax solution, which is later diluted to one-half with saturated borax, to which one-fifth proportion of eosin 0.5 per cent. in 70 per cent. alcohol is added. For my purposes I found a one-fifth to one-tenth proportion of the saturated methylene blue better, adding a few c.c. of eosin saturated in 95 per cent. alcohol. This stain was found the most satisfactory of all. It stains very rapidly, and shows the cytoplasmic and nuclear details with great sharpness, stains the pseudopodia well, the cytoplasmic bodies differentially, and fibrin faintly. Conklin's picro-hematoxylin is diluted Delafield's hematoxylin with a few drops of picro-sulphuric fixative added. It is slow in action, requiring an hour or longer; it is particularly good for the details of the cells, but best for the finer pseudopodia. It does not stain the leucocytes differentially and in time causes a precipitate. On the other hand, it stains the fibrin and small fragments of the blood.

On the whole, the hematoxylin and carmine stains when used directly are of little value for diagnostic purposes, while the aniline dyes serve excellently because of their differential staining. The foregoing stains were found effective in studying the corpuscles of *Aeshna, Libellula, Enallagma,* among Odonata; *Dytiscus, Hydrophilus, Leptinotarsa, Prionus,* and other Coleoptera; *Pieris rapae* and other Lepidoptera; *Dissosteira, Melanoplus* and various other Locustidae; *Belostoma, Notonecta* among Hemiptera; *Leptocerus* and *Phryganea* among Trichoptera; *Stratiomyia, Odontomyia,* Muscoid larvae, etc., among Diptera; and various sawflies and bees among Hymenoptera. In addition, specimens of various species were fixed, sectioned and stained as controls.

As far as the corpuscles are concerned, the sections do not show as fine materials as can be obtained by direct staining methods.

2. Classification of the Corpuscles.

Graber, Cuénot, Berlese, and others have attempted to classify the corpuscles variously according to structure, function, origin, and staining reaction, or a combination of these. Unfortunately, the classifications are mostly based on what is found in a single genus or family and less on comparative studies.

Berlese's classification, based primarily on Muscidae, is as follows: (1) true amebocytes, of embryonic origin, which serve to carry the plastic elements to the tissues; (2) splanchnocytes, smaller in size, with cytoplasm more homogeneous and more deeply stained, which pierce the intestinal coat to replace the intestinal epithelium after its destruction; (3) myocytes and sarcocytes, derived from the destruction of the larval muscles and recognized by their elongated nucleus, their homogeneous protoplasm and strong staining reaction to hemalum; (4) steatocytes, found in the Muscidae, cells which detach themselevs from the adipose tissue of the imagines, to destroy larval residues.

Cuénot's classification, based on Orthoptera, differs materially. He recognizes four types: (1) Amebocytes of small size, with large nucleus and compact cytoplasm, reproducing by mitosis (= myocytes of Voinov, and Berlese's No. 2). (2) Amebocytes of larger size, with abundant protoplasm surrounding a relatively small nucleus; these are the adult corpuscles; when they multiply, which happens rarely, it is by direct division, and one can find after this division masses of protoplasm containing several nuclei (Berlese, No. 1). (3) Other elements which have undergone a beginning of degeneration and contain in their protoplasm acidophil granules. (4) Cells in which degeneration is more advanced; here the protoplasm colors strongly and contains numerous debris of chromatin coming from a chromatolysis of the nucleus (sarcocytes of Berlese, No. 3).

Even from these two summaries it is evident that investigators differ in the interpretation of the various elements. Personally, I find two types of leucocytes prominent in adults and recognizable in the various species by both their size and staining reaction with aniline dyes: (A) Small or rounded corpuscles, somewhat spherical in repose, with a marked cytoplasmic affinity for aniline dyes; (B) larger corpuscles, resembling Amebae, flat or

spindular in repose, which react faintly with the same dyes. The latter are the so-called amebocytes. Type A is more embryonic in nature, and gives rise to a multitude of varieties with various functions. As a matter of fact, the amebocytes (type B) are derived from the smaller bodies, for one can find the transitions in size, shape, and staining reactions.

In the following classification the amebocytes are treated as separate entities, as a matter of convenience, since they are prominent in the blood, easily recognized, and about as abundant as the smaller leucocytes. I have reserved the name amebocytes for them. For the smaller bodies I propose the group name "chromophil leucocytes," because of their affinity for aniline dyes. There are other names which might have precedence, such as myocytes and splanchnocytes; but both of these terms apply to specific varieties with definite functions, and are therefore not sufficiently inclusive. Moreover, the term "myocyte" has been used rather promiscuously, so that it is misleading.

Classification of the Blood Corpuscles or Leucocytes. (Based on structure, function, and staining reaction).

1. Amebocytes—originate from the chromophil leucocytes; vary in size from one to four times the diameter of the chrom. leuc.; when floating they are hat-shaped or spindular, when active they are distinctly flat and highly ameboid, with many pseudopodia, some of them extremely long; nucleus relatively small, varied in shape; cytoplasm three to six times the diameter of the nucleus. Appear to divide amitotically. They stain very faintly with aniline dyes. Intermediates between chromophils are frequent. These are No. 2 of Cuénot, No. 1 of Berlese.
2. Chromophil leucocytes—embryonic in origin, and more or less embryonic in nature. Cytoplasm with marked affinity for aniline dyes. Somewhat rounded in shape both in repose and when active, and slightly flattened; pseudopodia many or few, always short; nucleus large, cytoplasm one-fourth to one-half its diameter. Divide mitotically. These are the myocytes of Voinov and others, Berlese, No. 2, Cuénot, No. 1. Various types can be recognized according to activity.
   a. Secreting chromophils—may be recognized by presence of cytoplasmic bodies, which tend to form a "terminal mass" at one end of the cell. The mass is secreted into the blood, and probably comprises histolytic and other enzymes. Form about one-third to one-half of the chromophils.

b. Transporting chromophils—generally with numerous small fat bubbles externally, especially during feeding periods. Frequently stained with plasmal pigments. Form about one-third of the corpuscles.

c. Phagocytic chromophils—corpuscles with engulfed or adsorbed tissue fragments.

aa. Small phagocytes, which appear like ordinary chromophils, but are studded with small fragments, which may stain deeply. More conspicuous in adults than in larvae.

bb. Granule spheres (Körnchenkugeln of Weismann). Recognizable by their increased size, up to five times the diameter of the amebocytes, "tomato"-like shape, and intensified staining reaction. Cytoplasm with numerous tissue fragments, which do not stain with direct stains; hence the cells appear vacuolated. Abundant in larvae, and in adults after ecdysis. These are enlarged phagocytes, and intermediates can be found readily.

d. Splanchnocytes (Berlese, No. 2)—cells which penetrate the intestinal coat to replace the intestinal epithelium. Not distinct from other chromophils, except that they are smallest in size, without external or internal bodies, and stain more intensely than most chromophils. Best recognized in sectioned material.

e. Degenerating leucocytes—generally of slightly larger size, with reduced or absent perinuclear space and acidophil granules. Cytoplasm stains unevenly. More evident in adults after ecdysis (No. 3 of Cuénot).

The myocytes of Berlese (No. 3) are strictly embryonic cells, with considerable resemblance to amebocytes. The nucleus is generally elongate, but may be round. Rare in the blood. In sections they are recognized by their different staining reaction—generally more intense.

The sarcocytes of Berlese are degenerating cells of muscular origin and not true corpuscles (see part 4 of this paper). Cuénot's No. 4 are of this type. The steatocytes of Berlese (No. 4) I cannot place. Cells resembling them are found in adults, but these are phagocytes. Moreover, Berlese's findings on the corpuscles have been much questioned and his results need further investigation.

The foregoing classification is based on studies in which the usual sequence, as noted above, was reversed, proceeding from adults to the pupae and larvae. Thus the work became largely comparative, both as to stages and species. On the whole, I have

found more homologies than I could expect among the representatives of the various orders. While the average size of the corpuscles varies both within and between species, perhaps as much as in Vertebrates, the general types are readily recognizable. Their embryonic nature, however, is very marked and it is doubtful if they should really be considered homologous with the leucocytes of higher forms. The plasticity of the chromophils is especially pronounced, as indicated by the studies of various investigators, and confirmed by my personal results.

3. Structure of the Corpuscles.

A. *Amebocytes.*

The amebocytes vary considerably in size both within an individual and in different species (Graber). They vary from cells as small as the chromophil leucocytes to as much as three times their diameter. Their outline is exceedingly variable (Figs. 1–4), and when active they remind one of various Amebae. When contracted or floating they are hat-shaped or spindle-shaped (Figs. 5–6), and their flat structure becomes apparent. The nucleus is generally oval, but frequently highly irregular, with indentations and protuberances (Figs. 2, 4). The nuclear contents are linin threads and chromatin granules, or chromosomes. A nucleolus is present, but not always evident; it is generally elongate in outline, less frequently rounded (Figs. 1, 2, 4).

The nucleus is surrounded by a clear perinuclear space (Fig. 1, ps), which is about one-fifth the diameter of the nucleus. Fine cytoplasmic threads penetrate this space to connect the nucleus with the cytoplasm. In dead or degenerating amebocytes this space is obliterated.

The cytoplasm consists of a thin external film of ectoplasm and a granular endoplasm. Berlese, Henneguy, and others call this cytoplasm homogeneous; on the contrary, I find it very granular (Fig. 1), with larger and smaller granules grouped irregularly, so that cells often appear "mottled." Occasionally, round or oval cytoplasmic bodies are present. Small vacuoles are abundant, and generally a few small fat bubbles. The latter can be recognized by their definite outline, and their homogeneous appearance. Since in some cells they stain deeply, in others not at all, it is evident that different series of fats are present.

The pseudopodia on the whole are rather fine. With Pianese's stain they show definitely. With most stains they appear to be made up of ectoplasm only, but picro-hematoxylin shows fine strands of endoplasm extending to the very tips. The pseudopodia vary in number; I have counted as many as sixty extending from the margins of an amebocyte and could not count those above and below. Figure 4 shows lappet-like pseudopodia, from the end of which extend finer fibrillae. These are quite common. The pseudopodia are thrust forth more abundantly in drops exposed to light than when kept in darkened chambers. In length they vary greatly; during coagulation of the blood pseudopodia of enormous length are extended, and I have observed cells with the lappets measuring as much as thirty times the length of the cell.

The amebocytes are highly thigmotactic. In transparent larvae, as *Enallagma, Chironomids, Dytiscus, Acilius,* and others, and in the hind wings of many adults they may be observed on the walls of the haemocoel and the wing channels, respectively. In open drops they attach themselves to the slide, to the surface film, and to clumps of chromophils and spread out their pseudopodia. In clotting they form a pseudopodial mesh-work, which is then contracted, helping to draw the lips of the wound together.

B. *Chromophil Leucocytes.*

With aniline dyes these leucocytes stain deeply, less markedly with hematoxylins and carmines. It is easy to overstain them because of the great affinity of the cytoplasm for dyes. Thus, with a very weak solution of methylene blue, 0.1 per cent. or even 0.01 per cent., they stain a deep blue, while the amebocytes stain faintly or not at all. The chromophils are rounded in outline, and somewhat spherical in shape, but distinctly flattened (Figs. 7, 10). The nuclear contents are similar to the amebocytes, and the clear perinuclear space is equally marked. The endoplasm is granular, the ectoplasm thinner and barely discernible. Since intermediates in size and staining reaction between the amebocytes and chromophils are not at all uncommon, it is evident that the latter give rise to the amebocytes, these being a specialized type. The varieties of chromophils are many; it is probable that intensive study will show even more types than listed in this paper.

a. Secretory chromophils.—These can be recognized by the tendency to form a "terminal mass" at one end of the cell (Figs. 7, 10). They are quite abundant, forming about a third to one-half of the total number of chromophils. Their pseudopodia are generally few in number and quite short.

b. Transporting chromophils.—The transporting leucocytes are studded with tiny fat bubbles and are frequently yellowish in color. They extrude great numbers of pseudopodia (Fig. 8), and often lappet-like pseudopodia (Fig. 9) recalling those of the amebocytes.

c. Phagocytic chromophils.—If a fixed slide is stained with a differential histologic stain such as Mallory's fuchsin-aniline blue-orange G., the phagocytes stand out with fair clearness. With the stain mentioned, the small phagocytes stain blue and show reddish particles in their cytoplasm or exteriorly, while the large phagocytes contain larger red fragments. These red particles and fragments are chiefly muscle fragments, products of histolysis.

In their number of pseudopodia the small phagocytes resemble the transporting chromophils. With them they form strings and clusters during the agglutination which accompanies the formation of a clot. With direct stains they show cytoplasmic inclusions heaped against the nucleus, usually on one side, and fragments on the exterior of the cell. In adults they can be recognized with little difficulty.

The large phagocytes, or granule spheres, are recognizable by their huge size, much larger than any amebocyte, their spherically flattened shape (tomato-like), and their intensified reaction with direct stains. They are greatly distended by their contents of muscle fragments. Intermediates between the small phagocytes and the spheres are not uncommon. They very rarely send out short pseudopodia; once gorged, they float passively in the blood streams. They are abundant in larvae, and in adults may be found for several weeks after ecdysis.

d. Splanchnocytes.—These are the smaller chromophils, not distinguishable from others, except that they stain somewhat more intensely. In favorable sections one may find them attached to the intestinal wall or piercing the intestinal coats. I have found them in sections of *Leptinotarsa, Pieris rapae,* and muscoid larvae.

e. Degenerating leucocytes.—With Pianese's stain these react erratically, showing acidophil (red) granules and bodies, frequently a broken or compact nucleus, while the cytoplasm stains in patches,—in spots deeply, in other places faintly. Vacuoles are more frequent and larger, and the perinuclear space is absent.

4. Other Structural Elements Found in the Blood.

a. *Muscle bodies* (Caryolytes, sarcolytes, sarcocytes, and myocytes). Besides the leucocytes various types of structures occur in the blood, particularly in larvae, but also in adults shortly after ecdysis. It is necessary to consider these, for at times they are very confusing to students. Of these, certain bodies connected with the histolysis of muscles are important. Reference has already been made to them in connection with the granules spheres; but a brief consideration of their origin will make their identity clear.

According to Kowalevsky, the granules spheres are concerned with the histolysis of the larval muscles. The latter, he states, are broken up by the leucocytes (Henneguy assumes an autolysis of the muscles, followed by an invasion by the leucocytes), the loosened fragments forming so-called *sarcolytes, i.e.,* broken muscle fibers, which are engulfed by the leucocytes. The latter enlarge and become the *granule spheres.* According to Berlese, the fragments are not digested, but carried through the pupal period; after this the leucocytes plasmolyze and set the fragments free, to serve as nutriment for the organs of the adult. Thus, he holds, the leucocytes are not phagocytic in nature. Henneguy, however, finds that the fragments are digested and that the spheres give rise to the primitive cells of the adipose tissue of the adult. This whole question needs more investigation; compare with what has been said under "phagocytes."

Berlese recognizes two types of granule spheres: (1) the *sarcolytocytes, i.e.,* cells carrying muscle fragments, of leucocytic origin, and (2) the *caryolytes, i.e.,* cells with nuclear fragments, or muscle fragments accompanied by a nucleus. In the caryolytes the nuclear fragments condense to a central mass, which breaks up into smaller compact nuclear bodies, each with its own cytoplasm and membrane. These smaller masses are later set free, and constitute the muscle cellules, or *sarcocytes.* Further, Berlese states, the sarcocytes transform into elongate, spindle-shaped cells, the nucleus grows larger, showing chromatin gran-

ules, while the cytoplasm is homogeneous. These are the *myocytes*, or muscle cells, which eventually build the muscles of the adult.

Henneguy questions this somewhat adventurous assumption of a degeneration of the caryolytes into sarcocytes and their transformation into myocytes. He believes, that the sarcocytes are due to a chromatolysis of the caryolytes and are eventually either absorbed by the hemolymph or ingested by the leucocytes. Personally, I believe that both processes take place, for quite generally one finds phagocytes studded with particles of various sizes adsorbed to the surface of the cell and other particles enclosed in the cytoplasm, and similar particles float freely in the blood, as shown very clearly in material stained with fuchsin-aniline blue-orange G. Such studded corpuscles are shown in the illustrations of Graber and Kolbe (see Packard). The particles also form the beads on fibrin threads.

The myocytes Henneguy considers present as embryonic cells which enter into the formation of the imaginal buds, or disks, dividing mitotically during nymphosis. These are joined by others which arise from the nuclei of the larval muscles and divide amitotically.

While the origin of these various bodies is of secondary interest in this study, the summary given will be of aid toward their recognition. On the whole, *caryolytes* are rare in the blood and react weakly with direct stains, if at all. They may be recognized by their two or more tiny nuclear fragments. *Sarcolytes,* or muscle fragments, are abundant, but take no direct stain and show no cellular organization. The *sarcocytes* may be excessively abundant, but are minute in size, with a tiny nuclear fragment. They stain erratically, with direct stains, like other degenerating tissue. They appear to undergo histolysis, and gradually break up. The *myocytes,* if present at all, are elongate or round, without pseudopodia, and resemble the leucocytes.

The non-corpuscular nature of these bodies can be more clearly demonstrated by fixation and staining with differential histological stains, such as Pianese's methylene blue-eosin, Mallory's fuchsin-aniline blue-orange G., and others. I have used the latter after fixation with Zenker's fluid on both sections and " smear " preparations. In these the amebocytes stain blue, the chromophils with blue nuclei and bluish pink cytoplasm, while the sarcolytes, sarcocytes, and caryolytes are all a brilliant red in

color. The few myocytes show a bright-red cytoplasm and purplish nuclei. Small and large phacocytes are blue, with the foreign inclusions or adhesions red. Since all the histolized muscular elements show a brilliant red without any blue, while the corpuscles are blue and bluish pink, confusion is obviated. Pianese's stain shows similar marked contrasts, depending somewhat on the type of methylene blue and eosin used; rectified or bacterial methylene blue is preferable, while yellow eosin stains less precisely than bluish eosin.

b. *Fat bubbles.*—Fat bubbles of varying size are frequent in the blood at all times. They are distinguishable by their circular shape, definite outline, " smooth " appearance, yellowish color, and strong refraction of light. Kolbe speaks of a " circular nucleus " for the fat bubbles. In this he was undoubtedly misled by the strong refraction, for careful staining with Sudan III or Scharlach Rot and counterstaining with hematoxylin shows the lack of any cellular elements. Due to the refraction of light, it often appears as if a nucleus were present, but the same may be observed in blood treated with concentrated sulphuric acid. The acid dissolves all the cellular elements, but not the fat. The bubbles float to the surface and, by refraction, still show " a dark, circular nucleus."

c. *Adipose Cells.*—The adipose cells are huge in size, round or elongate oval in outline, filled with numerous fat bubbles, and show blackish in transmitted light. They exceed the largest amebocytes by three to six or more diameters. With direct stains they behave erratically, portions staining diffusely or not at all. Their fat contents also stain unequally. In blood from pupae they are abundant, otherwise rare.

d. *Oenocytes.*—These cells are infrequent in blood obtained from punctures. When found, they are recognizable by their great size, rounded shape, round nucleus, and distinctly homogeneous cytoplasm. They stain deeply with direct stains, the cytoplasm with a " smoothness " lacking in any of the corpuscles. Only the granule spheres approximate them in size, but are not easily confused with them.

e. *Parasites.*—Stages of parasites are quite frequent, but are not easily mistaken for other structures. They comprise bacteria and coelomic Gregarines. Bacteria are more abundant in aquatic species, while the spores and gametic stages of coelomic Gregarines are quite common in both terrestrial and aquatic forms.

In general they are barely visible with high powers, are resistant to weak fixatives and stains, and keep up a peculiar twisting, whirling, or dancing motion, which is partly spontaneous, and partly due to plasmal currents. Adult Gregarina may occur, but are recognized without difficulty by their segments, gray color, and great size.

Parasitic Nemathelminthes will also occur in the haemocoel, but less frequently, chiefly of the genera *Gordius, Mermis,* and *Oxyuris. Gordius* and *Mermis* are macroscopic, from half an inch to several inches in length. *Gordius,* especially the younger stages, I have found in the haemocoel of various grasshoppers, larger aquatic Coleoptera, larger Chironomid larvae and pupae, in Trichoptera (*Phryganea, Neuronia,* and *Leptocerus*), and in mayfly nymphs. *Mermis* is pinkish in color, and was observed in the blood of Chironomids and in a mayfly nymph. *Oxyuris* is microscopic in size, and is generally regarded as an intestinal parasite. But since I have observed it in the wing channels of *Leptinotarsa, Dytiscus,* and *Hydrophilus* adults and also in abdominal blood from the same species, it is evident that the parasite either pierces the intestinal wall and enters the haemocoel, or is emptied into it during the intestinal histolysis of the pupal period.

f. *Blood Dust.*—Finally, with highest powers one may perceive in fresh blood exceedingly fine particles which for lack of a better name are here called " blood dust." Their nature I have been unable to determine. Perhaps they are histolytic remnants, perhaps secretions, or, like the hemoconiae or blood dust of Vertebrates, infinitesimal portions of disintegrated corpuscles. Like bacteria and the spores of Gregarina they are whirled about by plasmal currents, and form " dancing bodies."

5. Summary of Structures Found in the Blood.

The various structures which may occur in the blood of insects group themselves into two classes, (1) the leucocytes, and (2) tissue cells or products, and parasites. Recognition is based on structure and direct staining.

I. Leucocytes—all with a perinuclear space.
   1. Amebocytes—flat in form, of variable size, oval nucleus, and numerous short or long pseudopodia. Stain faintly with aniline dyes.

2. Chromophil leucocytes—spherical, but somewhat flattened, relatively uniform in size, few or many short pseudopodia. Stain deeply. Of varieties the granule spheres are noticeable for their huge size and intensified reaction with stains. Other varieties are the secreting, transporting, phagocytic, splanchnic and degenerating leucocytes.

II. Other bodies—all lacking a perinuclear space.

3. Sarcolytes—muscle fragments without nucleus. Do not stain.
4. Sarcocytes—muscle fragments enclosing nuclear fragments. Tiny in size. Do not stain or very faintly.
5. Caryolytes—contain several nuclear fragments. Variable in size. Lack a central nucleus and perinuclear space. Stain faintly.
6. Myocytes—much like chromophils at times, but rare in the blood.
7. Fat bubbles—round, uniform, and yellow. Much varied in size. Refract light and do not stain when in plasma. May stain within cells.
8. Adipose cells—elongate oval or round in outline, size larger than amebocytes. Contain many fat bubbles. Blackish in transmitted light. Stain unevenly.
9. Oenocytes—cells much larger than amebocytes, round, with round nucleus. Stain deeply and "smoothly."
10. Parasitic stages—bacteria, spores and gametes of coelomic Gregarina, occasionally adult Gregarina. Minute in size, and do not stain. Keep up dancing motion. Adult Gregarina very large, segmented, and gray in color. Parasitic Nematodes (*Gordius, Mermis, Oxyuris*) are found more rarely; recognizable by size, shape, and motion.
11. Blood Dust—minute particles found under highest powers.

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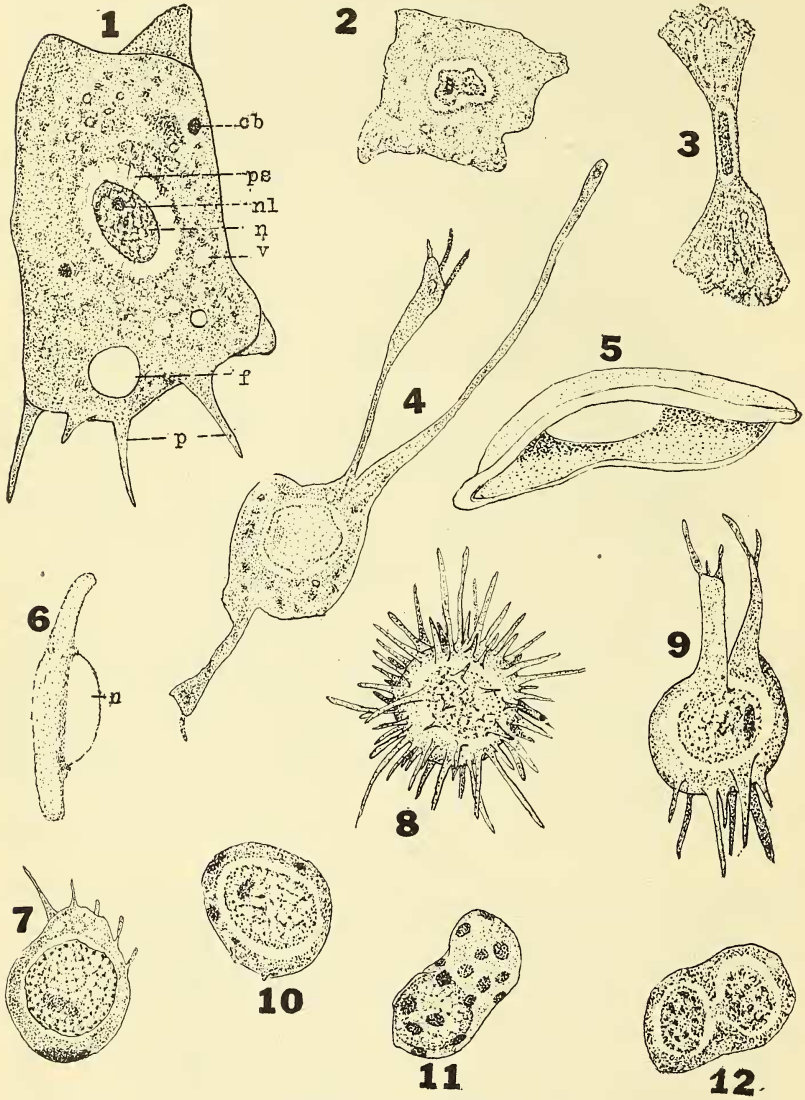

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Explanation of Figures.

1. Amebocyte, from *Dytiscus* larva. cb—cytoplasmic body, f—fat bubble, n—nucleus, nl—nucleolus, p—pseudopodia, ps—perinuclear speace, v—vacuole.
2, 3, 4. Amebocytes from *Leptinotarsa,* showing various forms. Fig. 4 shows lappet-like pseudopodia.
5, 6. Floating or contracted amebocytes, from *Aeshna* and *Dytiscus.*
7. Secreting chromophil leucocyte, from *Leptinotarsa.* tm—terminal mass.
8, 9. Transporting chromophils, showing pseudopodia. 8, from *Pieris rapae,* 9 from *Leptinotarsa.*
10. Secreting chromophil from *Enallagma.*
11. Degenerating chromophil (acidophil) from adult *Dytiscus.*
12. Dividing chromophil from adult *Hydrophilus.* The nuclei have reformed, but the cytoplasm is not yet divided.